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Report

Re-Evaluation of HBsAg Detection Kits Approved for Marketing in Japan

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INTRODUCTION

Approximately 1% of the Japanese population are estimated to be HBV (hepatitis B virus)-carriers. Rapid as well as accurate diagnosis of HB is extremely important from the viewpoint not only of medical treatment but also that of public health. Detection of HBsAg (hepatitis B virus surface antigen) in patient specimens has been widely used for diagnosis of HBV infection. In Japan, more than 40 different HBsAg detection kits approved by the Ministry of Health and Welfare (Ministry of Health, Labour and Welfare [MHLW], at present) have been commercially available for clinical use during the past two decades. It has become apparent that there are significant differences in sensitivity among the HBsAg kits currently on the market, mainly due to technical improvements made during the last 20 years. Under these circumstances, in order to provide medical personnel with up to date information on characteristics such as sensitivity and specificity, and to enable proper use of HBsAg detection kits, the National Institute of Infectious Diseases (NIID) has been requested to re-evaluate kits by various manufacturers/distributors according to the guidelines of MHLW. The present report aims at establishing an outline and results of the re-evaluation of HBsAg detection kits for release to the public.

METHODS

HBsAg detection kits evaluated in this study (Table 1)

In this study, we categorized HBsAg detection kits into four groups according to the principle/procedure used as follows: (A) Agglutination (manual), (B) Aggregation (automatic), (C) Immunochromatography, and (D) Enzyme immunoassay/Chemiluminescent enzyme immunoassay (EIA/CLEIA). Listed in Table 1 are product names, manufactures/distributors, assay objects, and sensitivities of HBsAg detection kits. This list includes kits that are to be commercially available in future; kits that have been withdrawn or are under consideration for withdrawal from the market; and kits whose "cut-off value" was under re-examination by manufactures/distributors as of September 2001.

Test procedure

Test samples utilized in the present re-evaluation tests were as follows:

① Negative samples (N): Eighteen HBsAg-negative sera samples, which were utilized at NIID for in-house qualification tests, and one HBsAg-negative sample (Accurun 1 multi-marker negative control serum) purchased from Boston Biomedica Inc. ([BBI], West Bridgewater, Mass., USA).

② HBsAg low titer panels: PHA-103 (13 samples) and PHA-105 (15 samples) purchased from BBI.

③ HBsAg mixed titer panels: PHA-203 (25 samples), PHA-204 (25 samples), and PHA-204M (21 samples; missing #11, #17, #23, and #24 in the PHA-204, all of which are indicated by an X in Table 2) purchased from BBI.

④ HBsAg seroconversion panel (SC): PHM-929 (9 samples) purchased from BBI. Sample numbers 1 to 9 indicate the bleeding order.

⑤ HBsAg National Standard (ST: 102 IU/ml) was diluted with the HBsAg-negative serum (BBI) to 16 IU/ml. The sample was then serially diluted by twofold with the BBI negative serum to the lowest concentration of 0.125 IU/ml.

For the two kits requiring manual operation, (A) Agglutination (manual) and (C) Immunochromatography, two low titer panels, PHA-103 and PHA-105, and two mixed titer panels, PHA-203 and PHA-204, and three other panels (① Negative, ④ Seroconversion panel, and ⑤ National Standard: see the results charts in Table 2) were used. For the agglutination/aggregation kits, confirmation tests utilizing anti-HBsAg antibody were performed on as many samples as were available, and the final judgments, positive versus negative, were described as confirmation test results.

For the two automatically operable kits, aggregation (automatic) and EIA/CLEIA, PHA-105 as the low titer panel, PHA-204 as the mixed titer panel, and three other panels (① Negative, ④ Seroconversion panel, and ⑤ National Standard) were used. Results were expressed as negative or positive. The judgment was based on the "cut-off values", which were described in manuals or calculated according to the formulas described in the manuals (Table 2, charts nos. 1 and 2).

RESULTS (Table 2)

Results obtained from screening tests for agglutination/aggregation, immunochromatography, and EIA/CLEIA kits utilizing the HBsAg panels 105 and 204 are shown in chart no. 1. Since some of the manually operated agglutination kits appeared to give false positive results, confirmation tests were performed when possible. With some agglutination kits, comprehensive judgments were not available in both screening and confirmation tests due to the agglutination of unsensitized control particles.

Results of confirmation tests utilizing panels 105 and 204 are shown in chart no. 2. These results indicated that the agglutination/aggregation kits and immunochromatography kits have a similar sensitivity. Although some low titer samples were judged as positive by some of the automatically operated aggregation kits, such results were not convincing given the sensitivities of the kits as evaluated by using National Standard HBsAg. Further, some of the kits judged some nega-

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Table 1.

(A) Agglutination (manual)

No.	Name	Manufacturer/Distributor	Detection of HBsAg in	Sensitivity
1	SERODIA-HBs	FUJIREBIO	serum/plasma	50-60 ng/ml
2	SERODIA-HBs・*PA*	FUJIREBIO	serum/plasma	30-40 ng/ml
3	Mycell HBsAg	Institute of Immunology	serum/plasma	5 I.U./ml
4	Mycell II HBsAg	Institute of Immunology	serum/plasma	5 I.U./ml
5	New Seroclit-HBs	Sanko Junyaku	serum/plasma	10 I.U./ml
6	Raphacell	MEGURO Institute	serum, plasma, or other blood specimen	50 ng/ml
7	QuickBeads HBsAg	SHINO-TEST	serum/plasma	4-8 I.U./ml

(B) Aggregation (automatic)

No.	Name	Manufacturer/Distributor	Detection of HBsAg in	Sensitivity
8	EXTEL HBsAg-H	Kyowa Medex	serum	3 ng/ml
9	Mycell Latex II HBsAg	Institute of Immunology	serum	5 I.U./ml
10	LPIA HBsAg Test G	Yuka Medias	serum/plasma	5 I.U./ml
11	RANREAM HBsAg	Sysmex	serum	0.5 U/ml
12	Runpia Latex HBs-Antigen	Kyokuto Pharmecut. Industr.	serum	5-8 I.U./ml
13	Mediace HBsAg	SEKISUI CHEMICAL	serum/plasma	5-8 I.U./ml

(C) Immunochromatography

No.	Name	Manufacturer/Distributor	Detection of HBsAg in	Sensitivity
14	Advanced Quality One Step HBsAg Test	Oriental Yeast	serum	5 ng/ml
15	ESPLINE HBsAg	FUJIREBIO	serum	5-10 ng/ml
16	Oligofast HBsAg NISSUI	NISSUI PHARMACEUT.	plasma	5 ng/ml
17	Dainascreen HBsAg	DAINABOT	serum, plasma and whole blood	2 ng/ml
18	Quick CHASER HBsAg	MIZUHO MEDY	serum/plasma	20 ng/ml
19	Bioclit-HBs	ADTEC	serum/plasma	3.2 I.U./ml

(D) EIA/CLEIA

No.	Name	Manufacturer/Distributor	Detection of HBsAg in	Sensitivity
20	IMMUNIS HBsAg EIA	Institute of Immunology	serum, plasma, or other blood specimen	0.63 I.U./ml
21	VIDAS Assay Kit HBsAg	BioMericux Japan	plasma	0.4-0.5 ng/ml
22	ELISA F-HBsAg	International Reagents	serum/plasma	0.2 I.U./ml
23	AIA-PACK HBsAg	TOSOH	serum	0.2-0.3 ng/ml
24	Monolisa HBsAg+	Bio-Rad Fujirebio	serum/plasma	0.1 ng/ml
25	PRISM HBsAg	DAINABOT	serum/plasma	0.2 ng/ml
26	ARCHITECT HBsAg	DAINABOT	serum/plasma	0.05 I.U./ml
27	AUSZYME MONOCLONAL	DAINABOT	serum/plasma	0.8 ng/ml
28	AxSYM HBsAg	DAINABOT	serum/plasma	1 ng/ml
29	Imx HBsAg	DAINABOT	serum/plasma	1 ng/ml
30	LUMIPULSE HBsAg	FUJIREBIO	serum	0.2-0.4 ng/ml
31	LPIA-F HBsAg Test G	Yuka Medias	serum/plasma	0.2 I.U./ml
32	Elecsys HBsAg	Roche Diagnostics	serum/plasma	0.024 U/ml
33	Cobas Core HBsAg EIA II	Roche Diagnostics	serum	0.062 U/ml
34	Enzygnost HBsAg 5.0	DADE BEHRING	serum	0.05 U/ml
35	SphereLight HBsAg	Wako Pure Chemical Industr.	serum/plasma	0.18 I.U./ml
36	EVATEST HBsAg	NISSUI PHARMACEUT.	serum, plasma and whole blood	1 I.U./ml
37	Lumispot 'Eiken' HBsAg	EIKEN CHEMICAL	serum/plasma	0.5 ng/ml
38	ORTHO Antibody to HBsAg ELISA Test System 3	Orhto-Clinical Diagnostic	serum/plasma	0.1-0.3 ng/ml
39	HBsAg REAGENT	KAINOS LABORATORIES	serum	0.48 I.U./ml
40	ACCESS HBsAg	BECKMAN COULTER	serum/plasma	0.5 ng/ml
41	AUTO ACE N HBsAg	AZWELL	serum/plasma	0.03-0.06 PEI.U./ml

tive samples as positive.

Samples that appeared to give false positive results as a result of the sensitivity of the kit utilized were subsequently submitted to confirmation tests. In chart no. 3, the results from both screening and confirmation tests for manually operated agglutination kits utilizing the other combination of HBsAg panels (103 and 203) are shown. In chart no. 4, the results of another panel (105 and 204), also shown in charts nos. 1 and 2, are shown again. Most agglutination/aggregation kits were able to detect around 1 ng/ml or 1 IU/ml HBsAg in samples. Samples that contained far less than 1 ng/ml or 1 IU/ml HBsAg were judged positive by some kits.

Two combinations of panels, 103/203 and 105/204, were utilized in two individual tests (charts nos. 3 and 4), while the same N, SC, and ST samples were utilized in both tests. Results differed between the two tests. Such ranges of test-to-test variation need to be taken into account when using these test kits. A portion of the results in charts nos. 5 and 6 are taken from charts nos. 1 and 2. These charts compare the results obtained by confirmation tests for agglutination kits with those obtained by immunochromatography kits. In general, manually operated agglutination kits and immunochromatography kits appear to have similar sensitivity. Our results also suggested some differences in sensitivity among kits within the same category.

DISCUSSION

Most of the HBsAg detection kits are designed for analyzing both serum and plasma samples, whereas 8 (nos. 8, 9, 11, 12, 23, 33, 34, 39) out of 41 kits are designed only for analyzing serum samples. BBI panel samples used here were mostly plasma samples, which may have led to the 8 kits generating somewhat different data. In addition, it should be noted that tests using automated kits (i.e., aggregation kits and most EIA/CLEIA kits) were performed only once.

Based on the results of the present re-evaluation, EIA/CLEIA kits seem to be better than agglutination/aggregation kits and immunochromatography kits in terms of general sensitivity and specificity. Among EIA/CLEIA kits, however, there were also substantial differences in sensitivities.

The study utilizing the National Standard HBsAg suggested that sensitivities of agglutination/aggregation kits and immunochromatography kits were approximately 4 IU/ml and 2 IU/ml, respectively, whereas those of EIA/CLEIA kits range from 1 IU/ml to 0.1 IU/ml. In addition to infectious particles (Dane particles), a large quantity of non-infectious particles was detected in the blood of HBV-carriers. For purposes of performing quantitative and qualitative analyses, it is important that HBsAg detection kits possess sensitivity suitable for the purposes, such as screening, diagnosis, monitoring, etc., of the test.

CONCLUSIONS

(1) Although it might be difficult to evaluate each assay kit simply by the results reported here, agglutination/aggregation kits and immunochromatography kits were, in general, inferior in sensitivity to EIA/CLEIA kits.

(2) According to the attached product document, when a sample is judged positive by a screening test utilizing an agglutination/aggregation kit, the sample must be further checked by a confirmation test and/or other more sensitive kits such as EIA/CLEIA in order to arrive at the final judgment.

(3) Diagnosis of HBV infection should not rely solely on results obtained by HBsAg detection kits. Final judgment should be made by incorporating data from other measures such as detection of anti-HBV core (anti-HBc) antibody and analysis of clinical status. In particular, when we use simple and easy but less sensitive assay kits such as agglutination/aggregation kits and immunochromatography kits, we must be conservative in evaluation of the test results.

(4) Differences in sensitivity were found among kits within the same category. In this respect, some revisions, including re-setting the "cut-off value", should be considered.

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APPENDIX

The members of the committee are as follows:

Toshitada Takemori (Chairperson), Hiroshi Yoshikura, Takeshi Kurata, Toshio Kishimoto, Ichiro Kurane, Nobuhiko Okabe, Katsutoshi Komuro, Masahiro Sakaguchi, Tetsuro Suzuki, Masato Tashiro, Shu-ichi Nakayama, Toshiaki Mizuochi, Tatsuo Miyamura and Namiko Yoshihara.

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Legends to Table 2.

a. Numbers 1 through 41 at the top of the charts indicate the serial numbers of the kits tested, and correspond to the numbers appearing in Table 1.

b. ▩: positive (+), ▨: weak positive (±), ☐: unable to be judged due to inexplicable agglutination in control groups, ◪: weak positive in screening without confirmation test, ⊠: unable to be judged because sample was missing in the panel.

c. The figures listed to the right of the panel numbers 105L and 204M indicate the HBsAg concentration (IU/ml) of each sample.

d. For panels 103L and 203M, the HBV subtype and HBsAg concentration (ng/ml) of each sample, mentioned in the BBI data sheet, are indicated in charts nos. 3 and 5.

e. (-) in panels 105L and 204M, and - in panels 103L and 203M represent HBsAg-negative samples.

In chart no. 1, the screening test results of agglutination/aggregation kits, immunochromatography kits, and EIA/CLEIA kits are listed. Due to several unascertained results (i.e., supposedly "false positives") obtained in the screening tests using manual agglutination kits, confirmation tests were employed (see charts nos. 2 to 4 for the results) except in the case of automatic aggregation kits.

In chart no. 2, confirmation test results of agglutination kits (manual) and screening test results of aggregation kits (automatic), immunochromatography kits, and EIA/CLEIA kits are listed. Results except for agglutination kits (manual) are the same as those shown in chart no. 1.

In charts nos. 3 and 4, results from both screening and confirmation tests of agglutination kits (manual) are listed for the purpose of comparison. Tests were done by using different panels as described earlier (chart no. 3: N/103/203/SC/ST, chart no. 4: N/105/204/SC/ST).

In charts nos. 5 and 6, confirmation test results of agglutination kits (manual) and screening test results of immunochromatography kits are listed for comparison. Results of agglutination kits (manual) are the same as those in charts nos. 2 and 4, and the results of immunochromatography kits are the same as those in charts nos. 1 and 2.

Table 2.

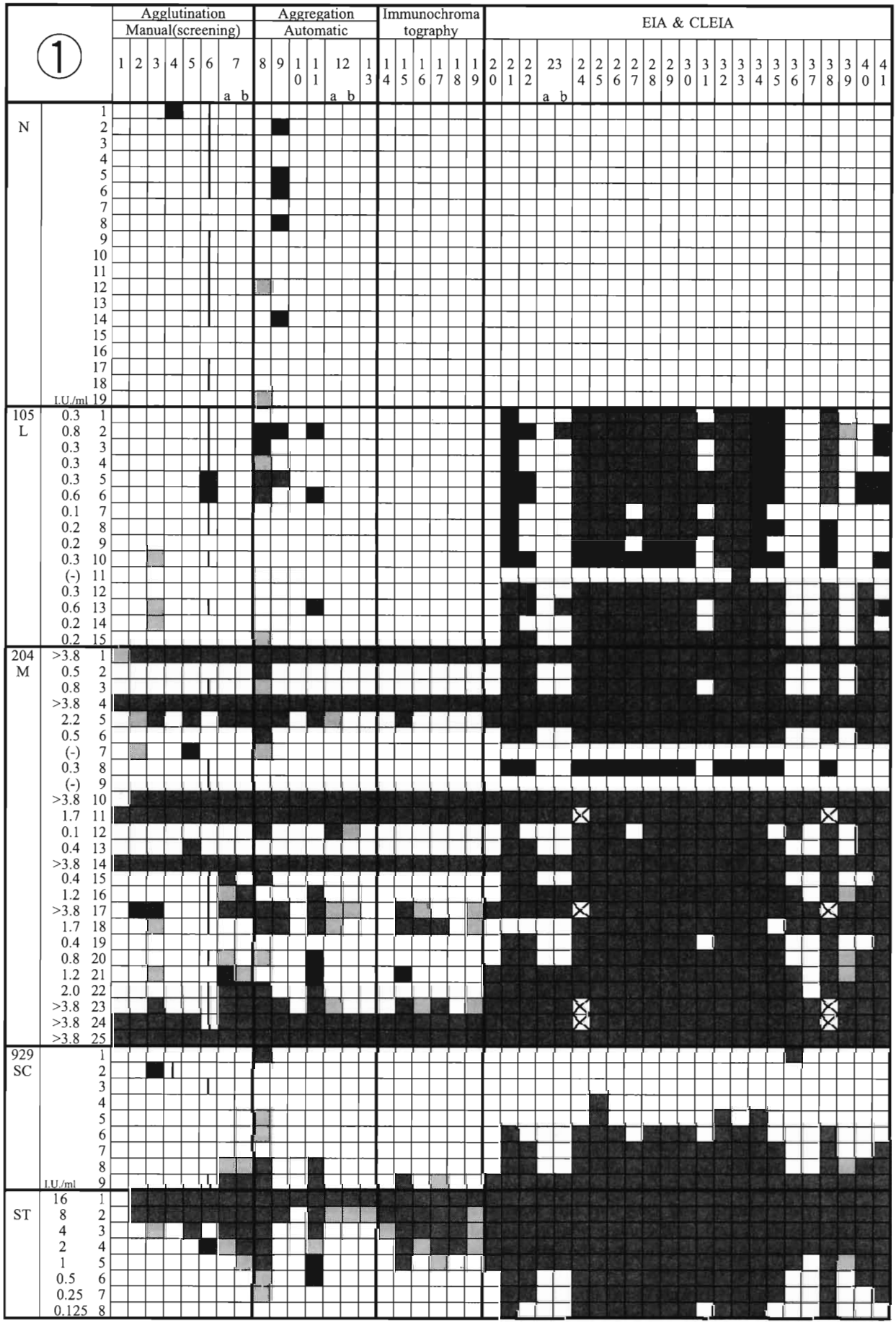
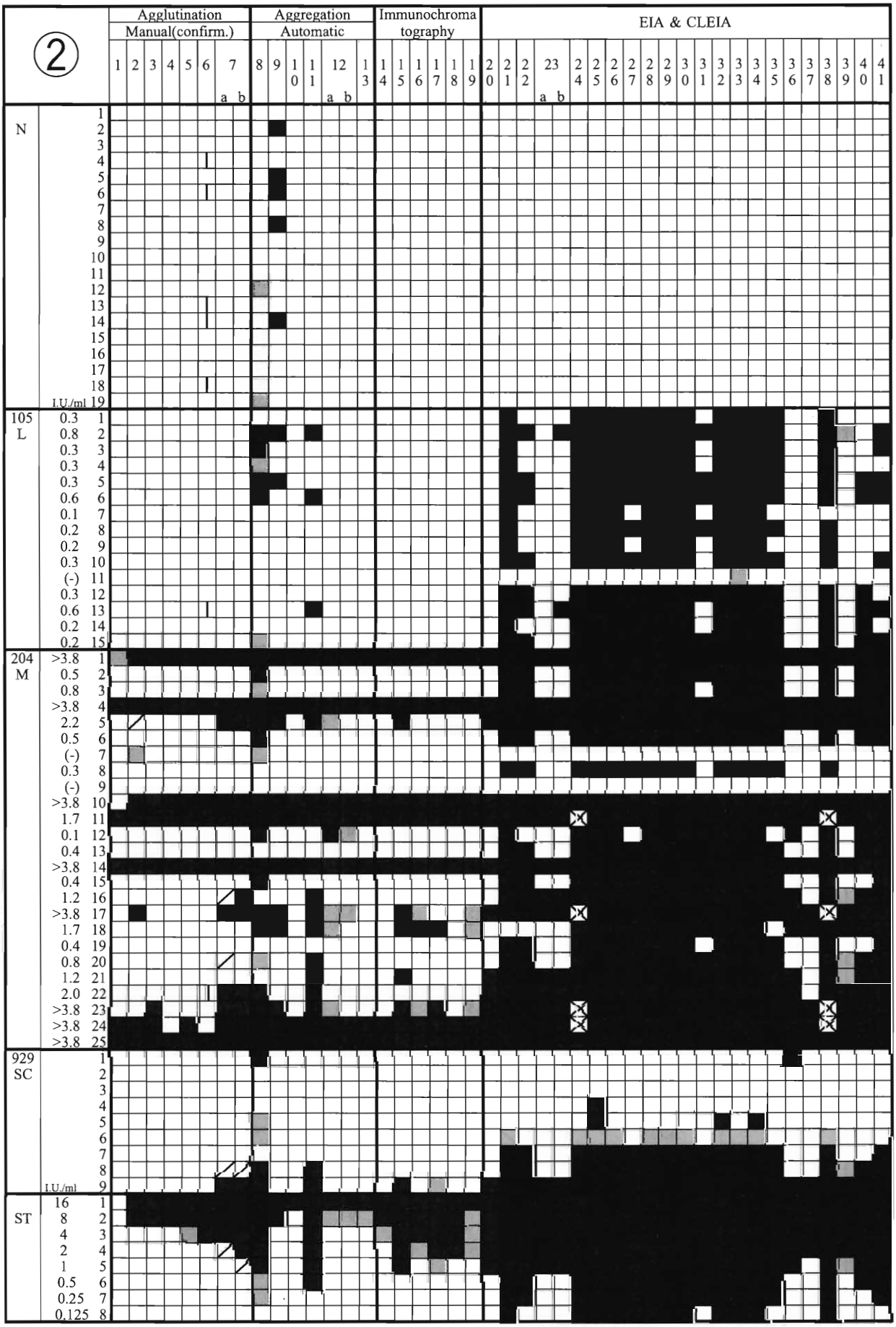

①
Agglutination
Manual(screening)
Aggregation
Automatic
Immunochroma tography
EIA & CLEIA
1 2 3 4 5 6 7 a b 8 9 10 11 12 a b 13 14 15 16 17 19 20 21 22 23 a b 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41
N
105 L
204 M
929 SC
ST
I.U./ml
16
8
4
2
1
0.5
0.25
0.125

Table 2-Continued.



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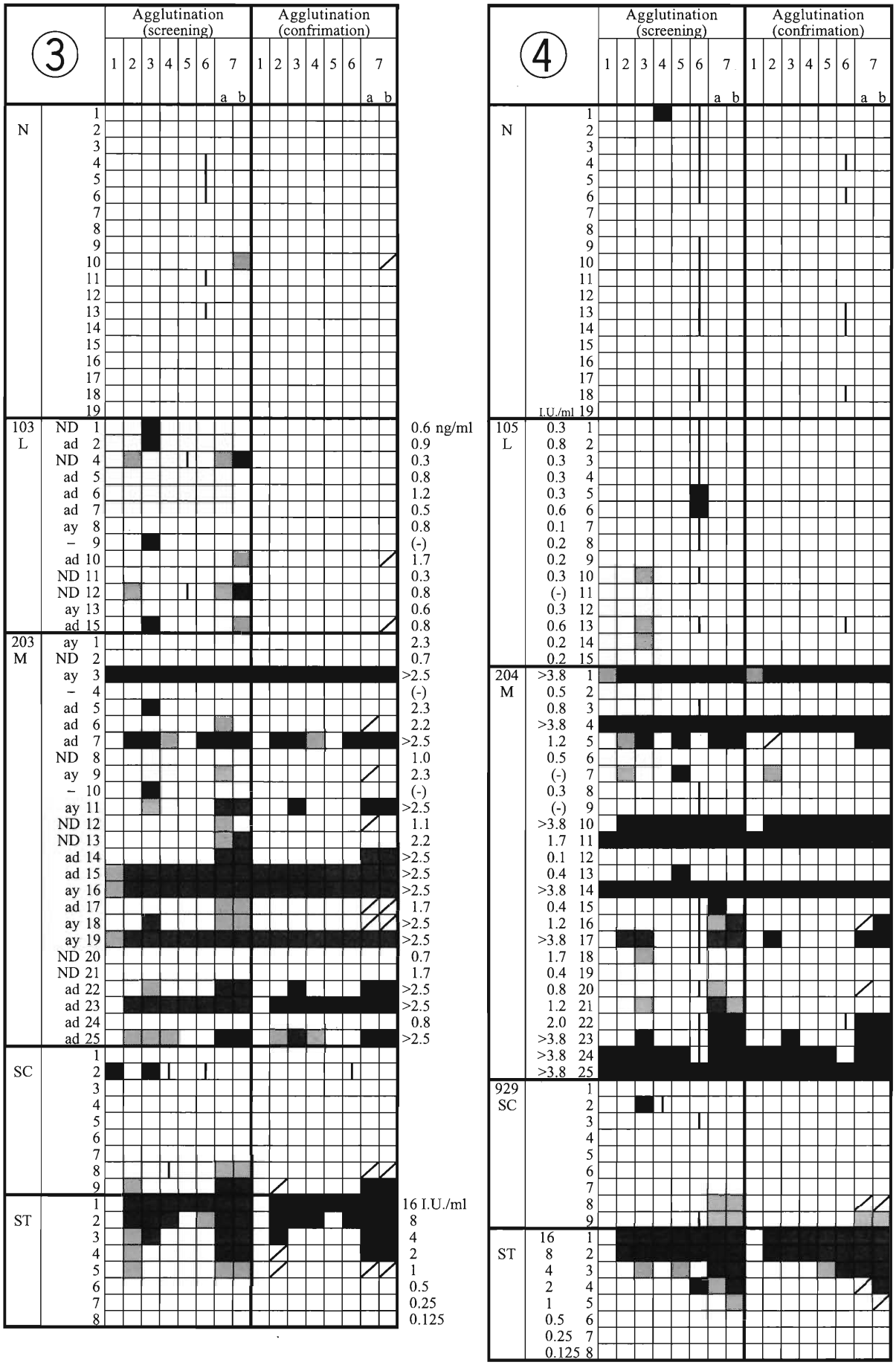

Table 2-Continued.